Original Article

Effects of Culture Medium and Concentration of Different Growth Regulators on Organogenesis Damask rose (*Rosa damascena* Mill)

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Citation Khajeh H, Fazeli F, Mazarie A. (2021). Effects of Culture Medium and Concentration of Different Growth Regulators on Organogenesis Damask rose (*Rosa damascena* Mill). *J. Plant Bioinform. Biotech.*, 1(1): 14-27

https://doi.org/10.22034/jpbb.2021.276335.1004

Article info
Received: 05 March 2021
Accepted: 09 May 2021
Available Online: 21 May 2021
Checked for Plagiarism: Yes
Peer Reviewers Approved by:
Bahman Fazeli-Nasab
Editor who Approved Publication:
Dr. Behzad Ghareyazi

Keywords:
MS, IBA, BAP, NAA, WPM

ABSTRACT

In vitro cultivation method is a proper way to fast propagation of commercial rose cultivars with high uniformity, and even today, it is regarded as a desirable solution for correction through genetic manipulation. In order to study the effect of hormone and culture medium on the micropropagation potential of GolD damask rose, an experiment was conducted as a completlely randomized design with three replications. In this study, Lateral bud explants, 5 culture media including MS, 1.2 MS, VS, 1.2 VS, and WPM with four plant growth regulators including benzylaminopurine (BAP), 2-isopentenyl adenine (2iP), naphthalene acetic acid (NAA), and indole butyric acid (IBA) were evaluated at concentrations of 0, 0.5, 1, and 1.5 mgL^{-1}. The results of analysis of variance showed a significant difference between culture media, type and causality of hormones, and their interaction for the studied traits. The best response for these traits was obtained from VS medium, and the weakest response was taken from WPM medium. The results of interaction effect showed that the highest values for shoot length, greenness, percentage of yellow leaves, fresh and dry weight of shoots in VS medium were obtained using BAP hormone at a concentration of 1.5 mgL^{-1}. The highest mean of rooting percentage, fresh and dry weight of roots was obtained in VS medium using 1.5 mgL^{-1} IBA.

1. Introduction

Damask rose flower (*Rosa damascena* Mill) is one of the most significant species of Wardsans. With a height of about one to two meters, deciduous shrubs have many flowering and prickly branches. This product is mostly planted in 11 provinces of Iran, among which Isfahan, Fars and Kerman provinces have the highest under-cultivation area and flower production [1]. The importance of Damask rose cultivation is its flower production. The essential oil and oil extracted from the petals are used in producing medicine and health cosmetics. Its dried petals, roses, and buds are also widely used in food industry. The classic and common methods of propagation of Damask rose are cuttings, root stocking, and grafting. Although propagation by vegetative organs is a dominant technique in most roses, the health and disease-free nature of plants obtained from these methods cannot be confirmed. Other limitations of such traditional

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ways are seasonal dependence, high reproduction costs, laborious work, and slow reproduction rates. Thus, using other methods of asexual reproduction that lead to production can be a good solution for these problems [2].

For years, applying micropropagation in plant propagation and production has attracted the attention of most researchers. This might be due to using traditional methods in plant propagation, especially globular plants which despite taking a lot of time can lead to the spread of diseases. Therefore, to produce healthy and uniform plants in short periods of time, it is necessary to use tissue culture methods. Plant tissue culture includes in vitro culture of plant components, i.e. tissue, organ, embryo, single cell, and protoplast, performed on nutrient culture medium and under sterile conditions. Each part of a plant is able to produce a complete embryo under suitable and special conditions. In laboratory cultures, all the chemical and physical needs of the plant organ must be met by the culture tube, the external medium, light, temperature, etc., and particularly, the culture medium [3, 4].

Culture medium is one of the most important components of plant tissue culture techniques and its successful application depends largely on culture mediums with right composition [5]. Different culture media vary greatly in ion composition and concentration. Ingredients of such media include mineral salts (high and low consumption elements) as a provider of maximum growth, amino acids and vitamins as nitrogen, sugar as a source of carbon and energy, agar as an aggregate of culture medium, and growth regulators as stimulate of growth, morphology and organogenesis [6]. One of the most important stages of micropropagation is the stage of branch propagation, since preparation of suitable shoots for rooting begins from explants which are used for mass propagation. Organogenesis in vitro depends on the use of plant hormones as well as the ability of tissues to respond to hormonal changes during culture [7].

Reproduction rate is affected by plant regulators, especially cytokinins. The presence of cytokinin helps to sample repoduction in culture medium. It plays a key role in cell division in plant tissues by controlling cell division and influencing factors that inhibit cell passage through cell division cycle. In addition to regulating the rate of division, this hormone also stimulates the growth of lateral buds. However, the amount of external regulators depends mostly on genotype and amount of internal hormone in plant. However, monitoring differentiation processes generally depends on presence of auxin and cytokinin, and their balance can lead to production of shoots and roots [8, 9].

During the rooting stage, the onset of roots in explants in vitro culture is a significant part of micropropagation. The ability of plant tissue to form aberrant roots depends on internal and external factors such as hormones. Much research has been done on effects of growth regulators of auxin group on rooting of cuttings in various plants. The most important synthetic auxins are indolebutyric acid and naphthalene-acetic acid, which are relatively more active than pure auxin of indol-acetic acid, and causes celluar division, lengthening, and growth, and stimulates formation of ectopic roots [10, 11]. Therefore, the aim of the present study was to investigate the effects of culture type, type and concentration of growth regulators on branching and rooting of Damask rose in in vitro conditions and to prepare optimal instructions for propagation of Damask rose shrubs in in vitro conditions.

2. Materials and Methods

In order to micropropagate *Rosa damascene*, a study was conducted in two separate factorial experiments in a completely random design with three replications in the tissue culture laboratory of Agricultural Biotechnology Institure, University of Zabol in 2020.

2.1. Preparation of Plant Samples

Suitable explants required for this experiment were taken from proper and full-flowered rootstocks of Damask rose flowers

grown in the research farm of University of Zabol. After transferring to pots, they were kept in the tissue culture greenhouse of the Agricultural Biotechnology Center until the beginning of the experiment. The explants obtained from single-node cuttings of the stem were placed under running water for 30 minutes after disinfection, and then were washed with a few drops of detergent. In all disinfection treatments, final rinsing of the explants was performed in sterile conditions under a laminar hood 3 times at intervals of 5, 10, and 15 minutes with sterile distilled water.

2.2. Experiment 1: Analysis of Effect of Medium, Type, and Concentration of Growth Regulators on Shoot Regeneration

In this experiment, MS, 1.2 MS, VS, 1.2 VS, and WPM medium containing 0, 0.5, 1, and 1.5 mgL^{-1} benzylaminopurine (BAP), 2 -Isopentenyl adenine (2iP) were used to proliferate rose explants. After 4 weeks, the desired growth indices such as shoot length, fresh and dry weight of shoots, percentage of greenness and yellowing of leaves were measured.

2.3. Experiment 2: Analysis of Effect of Medium, Type, and Concentration of Growth Regulators on Rooting

In the second stage, for in vitro rooting, shoots taken from MS, 1.2 MS, VS, 1.2 VS and WPM media with three different concentrations of Tallin acetic acid (NAA) and indole butyric hormone Acid (IBA) (control, 0.5, 1, 1.5 mgL^{-1}) were used. After 21 days, the desired growth characteristics (percentage of rooting, fresh and dry weight of roots) were measured.

After preparing the nutrient media of each experiment, their pH was adjusted to 5.7 and after adding agar at 121 °C, they were disinfected by autoclave. The incubation conditions used to maintain cultures in these experiments were 25±11 and 16 hours of light and 8 hours of darkness. Duncan's multiple range test was also performed.

Data related to different experiments in this study were analyzed using SAS ver 9.1 statistical software and the mean of treatments was compared with Duncan's multiple range test.

2.4. Data Analysis

Statistix 10 software was used for statistical calculations. Mean comparison was performed using the least significant difference (LSD) at the level of one percent and Excel software was also used to draw the shapes.

3. Results

3.1. Wet and Dry Weight of Air Organs

The results of analysis of variance showed that the effect of different levels of hormone, culture medium, surface concentration of 1% was significant and their dual effects on fresh and dry weight of aerial organs were at 5% level, but its triple interactions were not significant (Figure 1, Table 1). The results of comparing the mean on interaction of culture medium and hormone showed that by changing the type of culture medium and hormone, the fresh and dry weight of the aerial organ experienced a significant change, so that the highest fresh and dry weight of the aerial part was related to BAP hormone and VS culture medium (Table 2). Moreover, the results of the interaction in Table 3 showed that as a result of an increase in concentration of hormones, the fresh and dry weight of air organs had a rising trend, so that the highest fresh and dry weight of air organs was related to the concentration of 1.5 mgL^{-1} in BAP hormone. Based on the results of the interaction between culture medium and concentration, it was found that the highest amount of studied traits was related to VS culture medium with a concentration of 1.5 mgL^{-1} (Table 4).

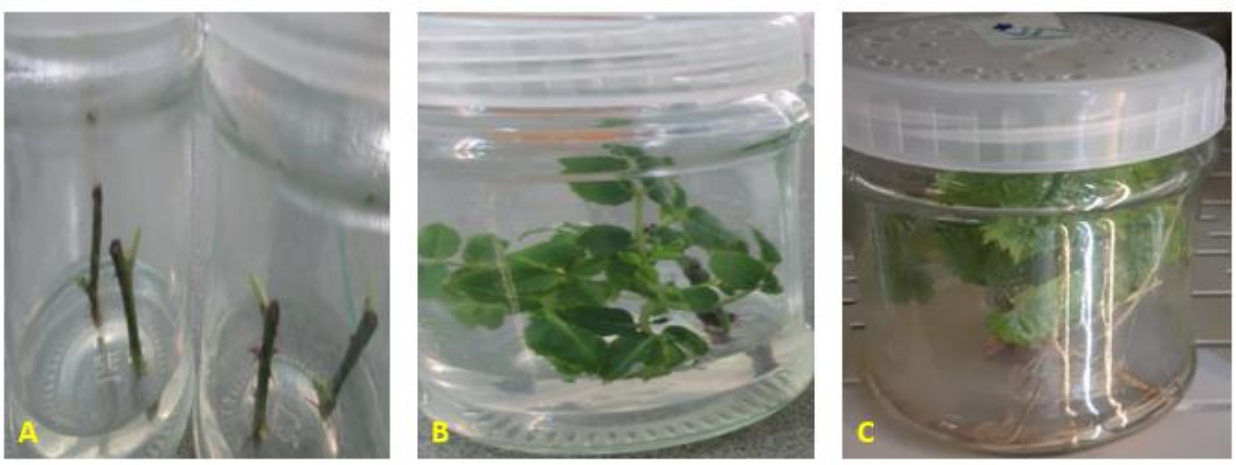

Figure 1. Shoots produced in VS medium and 1.5 mgL^{-1} IBA of *Rosa damascena* Mill.
(Planting of Lateral bud Explants (A), Branching (B), Rooting(C))

Table 1. Results of analysis of variance Effects of culture medium, hormone and concentration on some branching characteristics of Damask rose in in vitro conditions

Sources of changes: medium	Degree of freedom	Yellow leaf percentage	Stem length	Fresh weight of shoots	Dry weight of shoots
Medium	4	68242.73**	7.16**	0.312**	0/044**
Hormone	1	2398.14**	1.29**	0.327**	0.017**
Concentration	2	857.47**	0.192ns	0.069**	0.0045**
Medium*Hormone	4	1139.78**	1.25**	0.245*	0.015*
Medium* Concentration	8	243.04**	0.79**	0.021*	0.0044*
Concentration*Hormone	2	184.37**	0.255**	0.012*	0.0011*
Concentration*Hormone*Medium	8	135.01ns	0.499 ns	0.042 ns	0.0009 ns
Error	58	6.35	0.147	0.0107	0.00063
C.V.		7.13	9.97	6.52	5.32

3.2. Stem length

The results of analysis of variance showed that stem length was affected by different levels of hormone, culture medium, concentration and their dual effects, and the difference was statistically significant (%1), yet their triple interactions were significant (Table 1). The results of comparing the means in Table 2 showed that by changing the type of culture medium and hormone, the stem length could change significantly, so that the highest stem was related to BAP hormone and VS culture medium. However, the results of the interaction of hormone and concentration revealed that with increasing the concentration of hormones, stem length showed a more increasing trend than other levels, so that the maximum stem length was recorded in concentration of 1.5 mgL^{-1} BAP (Table 3). Based on the results of the interaction of culture medium and concentration, it was found that the highest value of the studied trait was related to VS culture medium and concentration of 1.5 mgL^{-1} (Table 4).

Table 2. Comparison of mean interaction of hormone type and culture medium on some branching characteristics of Damask rose

Treatments Medium	Hormone	Green leaf percentage	Yellow leaf percentage	Stem length	Fresh weight of shoots	Dry weight of shoots
BAP	VS	87.87a	21.4c	5.008a	2.05a	0.606a
	1/2VA	37.96c	24.79c	4.51a	1.61bc	0.504b
	MS	31.29cd	37.18b	3.79b	1.47de	0.448c
	1/2MS	25.19d-g	34.14b	3.7b	1.61bc	0.444c
	WPM	20.20fg	22.7c	2.84c	1.48c-e	0.433cd
2iP	VS	49.89b	23.47c	4.58a	1.51c-e	0.484b
	1/2VA	30.13c-e	22.25c	3.61b	1.63b	0.490b
	MS	28.57d-f	15.58d	3.73b	1.45e	0.489b
	1/2MS	19.8g	38.17b	3.36bc	1.56c-e	0.472cd
	WPM	22.51e-g	57.5a	3.37bc	1.46e	0.407d

The means with similar letters in each column do not have any significant difference.

3.3. Percentage of Green and Yellow Leaves

The results of analysis of variance showed that the percentage of green and yellow leaves of the plant was affected by different levels of hormone, culture medium, concentration and their dual effects. Also, the difference was statistically significant at the level of %1, but their triple interactions were not significant (Table 1). The results of comparing the mean on the interaction of the effect of culture medium and hormone showed that by changing the type of culture medium and hormone, the percentage of green and yellow leaves had a significant change so that the highest and lowest percentages of green and yellow leaves belonged to BAP hormone and VS culture medium, respectively (Table 2). On the other hand, the results of the interaction of hormone and concentration showed that with an increase in concentration of hormones, the percentage of green and yellow leaves had an increasing trend, so that the highest percentages of green and yellow leaves were related to the concentration of 1.5 mgL^{-1} BAP and 1.5 mgL^{-1} 2iP (Table 3). Also, based on the results of the interaction of Table 4, it was found that the highest and lowest values of the studied traits were related to VS culture medium and concentration of 1.5 mgL^{-1}.

Table 3. Comparison of mean interaction of hormone type and concentration on some branching characteristics of Damask rose

Treatments Hormone	Concentration	Green leaf percentage	Yellow leaf percentage	Stem length	Fresh weight of shoots	Dry weight of shoots
BAP	0.5 mgL^{-1}	33.75bc	24.3e	3.8bc	1.6ab	0.482ab
	1 mgL^{-1}	38.68b	26.39d	3.96b	1.61ab	0.485ab
	1.5 mgL^{-1}	49.08a	30.09bc	4.14a	1.72a	0.494a
2iP	0.5 mgL^{-1}	27.63d	31.89bc	3.76bc	1.5b	0.445bc
	1 mgL^{-1}	29.62c	32.2b	3.73bc	1.51b	0.454bc
	1.5 mgL^{-1}	33.28bc	33.61a	3.69c	1.55b	0.480ab

The means with similar letters in each column do not have any significant difference.

Table 4. Comparison of mean interaction effect of concentration and type of culture medium on some branching characteristics of Damask rose

Treatments Medium concentration		Green leaf percentage	Yellow leaf percentage	Stem length	Fresh weight of shoots	Dry weight of shoots
VS		61.5b	23.04cd	4.58b	1.73bc	0.502b-d
1/2 VA		32.81de	24.31cd	3.38d	1.55c-e	0.482b-e
MS	0.5 mgL^{-1}	17.27f	18.34e	4.02b-d	1.45e	0.459d-f
1/2 MS		23.34ef	25.24cd	3.7c-e	1.52c-e	0.452d-g
WPM		18.53f	44.62a	3.23de	1.5de	0.422fg
VS		70.51ab	25.07cd	4.74b	1.77ab	0.531b
1/2 VA		32.6de	22.43cd	4.18bc	1.58b-e	0/482b-e
MS	1 mgL^{-1}	25.1d-f	24.95cd	3.65c-e	1.47e	0.469d-f
1/2 MS		21.43ef	37.57b	3.57c-e	1.53c-e	0.433e-g
WPM		21.12ef	36.45bc	3.01e	1.44e	0.433e-g
VS		74.64a	19.2e	5.05a	1.84a	0.601a
1/2 VA		36.73cd	23.82cd	4.63b	1.73bc	0.527bc
MS	1.5 mgL^{-1}	47.42c	35.84bc	3.61c-e	1.44e	0.478c-e
1/2 MS		22.71ef	45.65a	3.33de	1.72b-d	0.423fg
WPM		24.41ef	39.24b	3.08e	1.46e	0.406g

The means with similar letters in each column do not have any significant difference.

3.4. Wet and Dry Weight of Roots

The results of analysis of variance showed that the effect of different levels of hormone, culture medium, concentration and their dual effects on some growth indices such as fresh and dry weight of roots were significant at the level of %1, but the triple interactions were not significant (Table 5). The results of comparing the mean on the interaction of culture medium and hormone showed that by changing the type of medium and hormone, fresh and dry weight of roots had a significant change, so that the highest fresh and dry weight of roots was related to IBA hormone and VS culture medium (Table 6). On the other hand, the results of the interaction of Table 7 revealed that with increasing the concentration of hormones, fresh and dry weight of roots had an increasing trend, so that the highest fresh and dry weight of roots was related to the concentration of 1.5 mgL^{-1} of IBA hormone. Moreover, based on the interaction effect of culture and concentration, it was found that the highest amount of studied traits was related to VS culture medium and concentration of 1.5 mgL^{-1} (Table 8).

Table 5. Results of analysis of variance of Effects of culture medium, hormones and concentration on some rooting characteristics of Damask rose in vitro

Sources of changes	Percentage of rooting	Dry weight of roots	Dry weight of roots	Degree of freedom
Medium	2135.7^{**}	0.031^{**}	0.004^{**}	4
Hormone	3383.68^{**}	0.073^{**}	0.007^{**}	1
Concentration	1098.27^{**}	0.083^{**}	0.0038^{**}	2
Hormone*medium	742.65^{**}	0.015^{**}	0.001^{**}	4
medium*concentration	263.04^{**}	0.006^{**}	0.00097^{**}	8
Hormone*concentration	23.02^{**}	0.15^{**}	0.0011^{*}	2
medium*Hormone*Concentration	330.3^{ns}	0.0058^{ns}	0.00061^{ns}	8
Error	47.3	0.00036	0.00024	58
Degree of Freedom	15.61	5.32	14.15	

ns, * and ** are no significant difference, significant difference at the level of %0.5 and %1, respectively

Table 6. Comparison of mean interaction of hormone type and culture medium on some rooting characteristics of Damask rose

Treatments		Rooting percentage	Fresh weight of roots	Dry weight of roots
Hormone	Medium			
	VS	78.41a	0.487a	0.150a
	1/2VA	45.49bc	0.385b	0.126ab
IBA	MS	40.89b-d	0.358b	0.107bc
	1/2MS	50.98b	0.397ab	0.116bc
	WPM	35.04cd	0.301b	0.091c
	VS	45.51bc	0.339b	0.116bc
	1/2VA	38.74cd	0.343b	0.095bc
NAA	MS	40.65b-d	0.353b	0.104bc
	1/2MS	35.04cd	0.316b	0.096bc
	WPM	29.56d	0.292c	0.089c

The means with similar letters in each column do not have any significant difference.

3.5. Rooting Percentage

The results of analysis of variance showed that the effect of different levels of hormone, culture medium, concentration and dual effects on rooting percentage was significant at the level of %1, but their triple interactions were not significant (Table 5). The results of comparing the mean on the interaction of culture medium and hormone also showed that the highest percentage of rooting was related to IBA hormone and VS culture medium (Table 6). On the other hand, the results of the interaction of Table 7 showed that with increasing the concentration of hormones, the percentage of rooting showed an increasing trend, so that the highest percentage of rooting was related to the concentration of 1.5 mgL^{-1} of IBA hormone. Based on the interaction effect of culture and concentration, it was found that the highest amount of studied traits was related to VS culture medium and concentration of 1.5 mgL^{-1} (Table 8).

Table 7. Comparison of mean interaction of hormone type and concentration on some rooting characteristics of Damask rose

Treatments		Rooting percentage	Fresh weight of roots	Dry weight of roots
Hormone	Concentration			
	0.5 mgL^{-1}	45.25bc	0.356b	0.113b
IBA	1 mgL^{-1}	49.44ab	0.384b	0.117ab
	1.5 mgL^{-1}	55.77a	0.417a	0.124a
	0.5 mgL^{-1}	30.97d	0.246c	0.082d
NAA	1 mgL^{-1}	38.08cd	0.346b	0.104c
	1.5 mgL^{-1}	44.64bc	0.393b	0.116ab

The means with similar letters in each column do not have any significant difference

Table 8. Comparison of mean interaction effect of concentration and type of culture medium on some rooting characteristics of Damask rose

Treatments		Rooting percentage	Fresh roots' weight	Dry roots weight
Medium	Concentration			
VS	**0.5 mgL^{-1}**	47.61cd	0.326bc	0.1c
1/2 VA	**0.5 mgL^{-1}**	36.56de	0.285bc	0.094c
MS	**0.5 mgL^{-1}**	35.37de	0.302bc	0.099c
1/2 MS	**0.5 mgL^{-1}**	35.1de	0.336bc	0.104bc
WPM	**0.5 mgL^{-1}**	35.92de	0.255c	0.09c
VS	**1 mgL^{-1}**	63.11b	0.412ab	0.141ab
1/2 VA	**1 mgL^{-1}**	40.39c-e	0.418ab	0.122abc
MS	**1 mgL^{-1}**	38.23de	0.359bc	0.102bc
1/2 MS	**1 mgL^{-1}**	49.81c	0.346bc	0.102bc
WPM	**1 mgL^{-1}**	27.32e	0.292bc	0.084c
VS	**1.5 mgL^{-1}**	75.17a	0.502a	0.158a
1/2 VA	**1.5 mgL^{-1}**	49.39c	0.389abc	0.116bc
MS	**1.5 mgL^{-1}**	48.7c	0.405ab	0.117bc
1/2 MS	**1.5 mgL^{-1}**	44.11cd	0.387abc	0.112bc
WPM	**1.5 mgL^{-1}**	33.65de	0.342	0.097c

The means with similar letters in each column do not have any significant difference

4. Discussion

In order to culture the plants of rose family, mostly Murashige and Skoog (MS) andVan der salm (VS) media and in a few cases, Schenk and Hilderbrandt (SH) and Woody plant medium (WPM) media were used [12]. Based on the results of this study, it was found that there were significant differences between MS, 2.1 MS, VS, 1/2 VS and WPM media. Also, several differences were reported in type and amount of some low-consumption and high-consumption elements and compounds, so the differences in the in vitro yield of the Damask rose refer to these differences as well. These results are in line with the findings of Moradian *et al*. [12], Imani Rastaei *et al*. [13], and Eghbali Sharhjini *et al*. [14]. In distinct experiments, the researchers compared the effects of two MS and VS media, five DKW, WPM, B5, L2, MS, N6 media, and four QL, VS, MS and NN media, respectively, and concluded that there were significant differences between these different media. This result also confirms the results of the present study.

Accordingly, in comparison with MS and WPM medium, FeEDTA iron was removed and replaced by FeEDDHA in VS medium. Also, potassium nitrate was removed and replaced by potassium sulfate in WPM medium compared to MS and VS medium, while the amount of Sulfate ion in WPM medium was 4.4 times higher than its value in VS and MS medium. Other differences between the three studied media were their nitrate and ammonium contents, which are added to the culture as the main source of nitrogen supply. Although the ratio of nitrate to ammonium was almost equal in the three media, their values in the media varied greatly, so that the nitrate content in MS and VS is 3.83 times higher than in WPM, and their ammonium content is 4.12 times higher than WPM.

According to Moradian *et al*. [12], Bolandi *et al*. [15], and Saadat *et al*. [16], in analyzing the effect of type of culture medium on shoot length, the best type of base medium on the proliferation of Damask rose flower specimens was VS medium (MS containing FeEDDHA iron chelate), so that VS medium significantly increased shoot length. The results of this experiment are in line with those of researchers working on Chinese marshmallow [17], bitter almond [18], and rose [19]. Research results on Chinese marshmallow [20] and two species of Rosa, beggeriana and Rosa Canina [12] showed that the highest mean branch length was obtained in MS medium with FeEDDHA (VS) compared with MS with FeEDTA [17]. Also, in a

study conducted by Bayanati *et al*. [21] on the species *Rosa hybrid cv. Black baccarat*, it was reported that since VS is superior to MS and WPM, it is the best culture for breeding this species, so that the highest and lowest branch heights were related to VS and WPM culture medium. In another report, it was stated that VS medium was superior to MS and WPM in all evaluated indices, whereas WPM culture medium had the lowest value in all studied indices, mainly due to its higher ionic strength [17].

Evaluation of the effect of culture medium type on some growth indices such as fresh and dry weight of roots and aerial parts showed that the explants grown first in VS and then in MS medium had the highest mean compared to other media. These findings were in line with those of Moradian *et al*. [12] and Zarei *et al*. [22] showing that the explants grown in VS and MS medium are superior to other media. Also, Zarei *et al*. [22] investigated the effect of different culture media including MS and WPM on some Gisela 6 basic growth indices and concluded that MS nedium was superior to WPM and other experimental mediums. Therefore, in analysis of important growth characteristics, this medium had a better performance, which in turn, confirms the results of the present study.

However, in studies conducted by Feizi *et al*. [20], the effect of VS, WPM and MS mediums weas investigated on Chinese marshmallow and it was reported that there is a significant difference between the studied mediums. Also, in terms of all growing indicators. VS medium was superior to WPM and MS, which confirms the results of this study showing superiority of VS medium to other media. Likewise, in another study done by Eghbali *et al*. [14] on VS medium and several other culture media, it was found that VS medium had the highest weight and dryness of shoots compared to NN and MS media.

One reason for this superiority is presence of FeEDDHA iron along with most ammonium, nitrate and chlorine ions [19]. Fe-EDDHA iron chelate increases the solubility of iron in the plant growth medium in vitro to provide more iron to the plant (Feizi *et al*., 2015). Increasing the concentration of trace elements such as Fe in the culture medium causes better access to nutrients and in turn, increases Fe in leaves and plants. The more the Fe content in leaves, the more the leaf chlorophyll. Clearly, supplying required iron in Chlorophyll structure leads to an increase in photosynthesis and materialization which consequently, increasing photosynthesis and materialization provides more dry matter [23]. On the other hand, in pH < 5.5, Fe-EDDHA preserves its solubility and provides more iron to the plant, so an increase in vegetative growth of the plant is obtained [24]. Another role of iron, which increases vegetative growth is nitrogen fixation.

Since nitrogen is involved in the synthesis of some enzymes, its levels have an immediate effect on cell division and plant growth. In other words, photosynthesis is influenced by the amount of nitrogen in plants [25]. Therefore, as mentioned earlier, the higher fresh and dry weight of shoots and roots in VS culture medium may be due to the presence of large amounts of nitrogen, especially nitrate, as well as an iron source in the form of FeEDDHA, because an increase in iron level can facilitate photosynthetic electron transfer, increase CO_2 stabilization, and improve concentration of starch and soluble carbohydrates in plants during the growing season, and finally, enhance production of dry matter in the plant.

In addition to the effect of culture medium on performance of plant micropropagation traits, hormones are also a key part in cell stimulation, bud formation and proliferation, as well as induction of rooting and their growth [15]. Based on the results of this study, all growth indices were affected by different plant growth regulators and their concentrations, and there was a significant difference between various concentrations of growth regulators and their type on some shoot regeneration characteristics so a significant increase in shoot length, freshness, fresh and dry weight of shoots was recorded along with an increasing concentration of BAP and 2iP, and the highest shoot length and percentage of greenness, fresh

and dry weight of shoots was recorded during application of 2 mgL^{-1} BAP. These results are also in consistent with the findings of other studies conducted by Feizi *et al*. [20], Safarnejad *et al*. [26], Hajian *et al*. [27] in which during the application of BAP hormone with increasing concentration, fresh and dry shoot weight, greenness, and shoot length ratio were more than those in control group.

The presence of cytokinins in culture medium is necessary for plant regeneration in vitro, because these hormones are essential for expression of genes which are suitable for stem bud differentiation [28]. The ability of different cytokinins to induce shoot length can be attributed to factors such as stability, motility, and rate of hormone synthesis and oxidation, so it seems that hormonal conditions can be the determining factor for successful growth of ectopic bud formation and shoot proliferation [29].

Rooting in cuttings has a very complex nature, and many internal factors, including hormonal treatments, are involved in its controlling [30]. Applying hormonal treatments, especially auxins, to induce rooting in *in vitro* seedlings and to provide enough roots of suitable size and without callus formation with good ability after establishment in soil is also significant. According to Sabatini *et al*. [31], the differentiation of root primers from phloem parenchymal cells depends on the type and concentration of auxin which is used. In this way, they are able to respond to organogenic signals [32].

Based on a study by Mansseri-Lamrioui *et al*. [33], the effect of different levels (0.5, 1, 2, 4 mg/l) of IBA, IAA and NAA auxins on rooting of wild cherry shoots showed different concentrations of IBA which performed better than other auxins and could produce the highest number of roots and the longest root length. On the other hand, high concentrations of NAA and IAA stopped root growth and hindered the rooting rate. Moreover, in another study by Feizi *et al*. [20], the effect of 0, 0.1, 0.2, and 0.4 concentrations of IBA and NAA hormones on the rooting of Chinese marshmallow was evaluated to show that different concentrations IBA had better yields than NAA levels, and the highest percentage of rooting and root length was related to the concentration of 0.2 mg/l IBA. Yet, in another report provided by Grossi et al. [34], the effect of IBA and NAA growth regulators on rooting traits of Qazvini pistachio was studied and it was found that the percentage of rooting and the length and number of roots in the presence of NAA were much higher than that of IBA. This finding was contradictory to that of the present study.

The results indicating the best rooting percentage obtained during the 2 mg/l IBA treatment were also is in line with the results of this study. In a study by Shojaei *et al*. [35], the best hormonal combination of IBA and NAA for rooting of Damask rose cultivar Kashan 2 was introduced in which 1 mgL^{-1} treatment had the highest rooting index. In another study by Babaei *et al*. [36] and Heidarpour *et al*. [30], the effect of different concentrations of IBA hormone on rooting of Ficus benjamin Ablaq and shrub was evaluated and it was concluded that increase in IBA concentration, rooting percentage, and the weight of wet and dry roots was greater than in control surfaces. This is also in consistent with the results of the present study.

Based on the results of this study, it was found that IBA rooting hormone had a better performance than NAA hormone. This finding was in line with the results of further studies done on rooting and root quality in cuttings of different plants showing that growth regulator IBA is more effective and efficient than NAA [37]. Non-toxicity of IBA at high concentrations [38], lack of its degradation by indole acetic oxidase, its slower photodegradation compared with the rapid photodegradation of IAA in tissue culture medium, IBA slow movement within tissue as well as its late destruction [39] may be some main reasons for the better performance of IBA than IAA.

By stimulating root formation, carbohydrates and nitrogenous substances are

transferred from the leaf to the root, which in turn, increase the weight of the root [40].

5. Conclusion

Propagation, proliferation, and rooting of rose shoots in *in vitro* culture are influenced by a great number of factors such as species, genotype, cultivar, culture medium, mineral salts, organic matter, carbohydrates, growth regulators, and mediumal conditions. Among these factors, the ratio of auxin concentration to cytokine and the type of culture medium is the most significant one. Comparing different culture media for Damask rose flower cultivation showed that VS culture medium is superior to solid culture medium, so an increase in all growth indices was recorded in the regeneration stage. Of compounds used to induce shoot regeneration, the combination of 1.5 mgL^{-1} BAP has the highest regenerative ability and the most suitable combination to induce shoot rooting is 1.5 mg per liter of IBA. Therefore, based on the results of this study, to induce rooting and regeneration of the Damask rose shoots, it is suggested to use VS culture medium along with simultenous application of different concentrations of BAP and IBA, since it is possible to have greater increase in terms of growing indices and dry matter.

Conflict of interest

The authors declare no conflict of interest.

Consent for publications

All authors read and approved the final manuscript for publication.

Availability of data and material

The authors declare that they have embedded all data in the manuscript.

Ethics approval and consent to participate

No human or animals were used in the present research.

Author contributions

All authors read and approved the manuscript.

Acknowledgments

The authors thankfully confess for the funding of University of Zabol.

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